Published in final edited form as:

N Engl J Med. 2009 February 12; 360(7): 659–667. doi:10.1056/NEJMoa0806122.

B-Cell Clones as Early Markers for Chronic Lymphocytic Leukemia

Ola Landgren, M.D., Ph.D.,
Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD

Maher Albitar, M.D.,
Quest Diagnostics, Nichols Institute, San Juan Capistrano, CA

Wanlong Ma, M.S.,
Quest Diagnostics, Nichols Institute, San Juan Capistrano, CA

Fatima Abbasi, M.S., M.P.H.,
Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD

Richard B. Hayes, Ph.D.,
Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD

Paolo Ghia, M.D., Ph.D.,
Unit and Laboratory of Lymphoid Malignancies, Department of Oncology, Università Vita-Salute San Raffaele and Istituto Scientifico San Raffaele, Milan

Gerald E. Marti, M.D., Ph.D.,
Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD

Neil E. Caporaso, M.D.
Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD

Abstract

BACKGROUND—Otherwise healthy persons with a small number of B-cell clones circulating in the peripheral blood have been designated as having monoclonal B-cell lymphocytosis (MBL). Hospital-based series indicate an excess risk of progression from MBL to chronic lymphocytic leukemia (CLL). In this prospective cohort study, we tested the hypothesis that CLL is always preceded by MBL.

METHODS—Among 77,469 healthy adults who were enrolled in the nationwide, population-based Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, we identified 45 subjects in whom CLL was subsequently diagnosed (up to 6.4 years later) through the collection of a peripheral-blood sample. Using six-color flow cytometry (with antibodies CD45, CD19, CD5,

Address reprint requests to Dr. Landgren at the Center for Cancer Research, National Cancer Institute, Bldg. 10, Rm. 13N240, 10 Center Dr., Bethesda, MD 20892, or at landgreo@mail.nih.gov.

CD10, kappa, and lambda) and immunoglobulin heavy-chain gene rearrangement by reverse-transcriptase–polymerase-chain-reaction assay, we determined the association between MBL and subsequent CLL and characterized the immunoglobulin gene repertoire of the prediagnostic B-cell clones.

RESULTS—On the basis of either flow-cytometric or molecular analysis, 44 of 45 patients with CLL (98%; 95% confidence interval [CI], 88 to 100) had a prediagnostic B-cell clone; in 41 patients (91%; 95% CI, 79 to 98), the presence of the B-cell clone was confirmed by both methods. The presence of immunoglobulin heavy-chain variable (*IGHV*) genes was determined in 35 of 45 prediagnostic clones (78%). Of these clones, 16 (46%) were *IGHV3* subgroup genes (including 6 [17%] *IGHV3–23* genes) and 9 (26%) were *IGHV4* subgroup genes (including 4 [11%] *IGHV4–34* genes). Furthermore, 27 of 35 of the *IGHV* sequences (77%) had mutations, with similar distributions after stratification either below or above the median time between the collection of the prediagnostic blood sample and the subsequent CLL diagnosis.

CONCLUSIONS—In peripheral blood obtained up to 77 months before a CLL diagnosis, prediagnostic B-cell clones were present in 44 of 45 patients with CLL.

CHRONIC LYMPHOCYTIC LEUKEMIA (CLL), the most common leukemia among adults in Western countries,[1] is characterized by an accumulation of mature B cells.[2] Although male sex, advanced age, white race, and a family history of CLL or other lymphoproliferative cancers are recognized risk factors,[3,4] the cause and pathogenesis of CLL are largely unknown. Genetic factors are important in the development of CLL on the basis of evidence from multiply affected families, case series, and registry studies of twins and case–control and population-based subjects.[1–11] Also, findings of an association between polymorphisms and an increased susceptibility to CLL support a role for genetic factors.[5,6] Several lines of evidence suggest a role for antigenic stimulation in the development of CLL.[7–13]

Using flow cytometry, investigators have identified small B-cell clones with a surface phenotype similar to that of CLL circulating in the peripheral blood of healthy adults who have no evidence of other lymphoproliferative disorders.[14–22] Although many terms have been used to describe this condition, an international consensus has recommended monoclonal B-cell lymphocytosis (MBL), which indicates the presence in the blood of monoclonal B cells in numbers below 5000 per cubic millimeter.[17] The prevalence of MBL reportedly ranges from 3 to 5% in the general population over the age of 50 years.[19,21–23] With the exception of an increasing frequency of MBL with older age[24] and in first-degree relatives of patients with CLL,[16,19,25] population subgroups at risk for MBL have not been identified. Small, hospital-based case series from the United Kingdom have indicated that patients with MBL who have increased levels of monoclonal B cells show a steady increase in disease levels over time, with about 5% progressing to clinically recognizable CLL (including 1% per year who require chemotherapy).[23,26] Although it is plausible that MBL confers an increased risk of CLL, the precise degree of risk has not been studied, since no group of patients with MBL has yet been prospectively identified and followed.

A key gap in our understanding is whether CLL is always preceded by MBL, whether CLL typically arises de novo, or whether both processes occur. Taking advantage of the large

nationwide Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial,[27] we conducted a prospective study to address these questions. Among more than 77,000 subjects who were cancer-free at baseline, we identified all those in whom CLL was subsequently diagnosed. Using stored prediagnostic peripheral-blood samples obtained before the CLL diagnosis, we aimed to define the association between MBL and CLL and to characterize the immunoglobulin gene repertoire of the prediagnostic B-cell clones.

METHODS

STUDY POPULATION

The subjects in the PLCO Cancer Screening Trial have been described previously.[27] Briefly, from 1992 through 2001, more than 150,000 subjects between the ages of 55 and 74 years from 10 study centers across the United States were randomly assigned to undergo a specific cancer-screening regimen (screening group) or receive routine medical care (control group) to evaluate the effects of screening on disease-specific mortality. At baseline, all study subjects provided written informed consent and completed a questionnaire regarding demographic and risk-factor characteristics.[28]

We drew our study population from the 77,469 subjects in the screening group. Initially, we identified 129 subjects with incident CLL in the database; of these subjects, 123 had given their consent to participate in research studies. Forty-six of the 123 subjects had available prediagnostic cryopreserved whole blood. For one subject, the stored blood contained an insufficient number of cells for our analysis. Therefore, 45 subjects with CLL were eligible for our study. For all subjects, a blood sample had been obtained at least 3 months before the date of diagnosis. We also included 10 subjects without a CLL diagnosis as negative controls.

Subjects in the screening group of the PLCO Cancer Screening Trial had undergone testing for the detection of prostate cancer (the prostate-specific antigen [PSA] test and digital rectal examination), lung cancer (chest radiography), colorectal cancer (sigmoidoscopy), and ovarian cancer (the CA-125 test and transvaginal ultrasonography). Subjects who provided annual blood samples for 6 years for the PSA test (in men) or CA-125 (in women) were also asked to contribute additional blood samples for research on cancer and other diseases common to their age group. In addition, information on incident cancers (type and date) was obtained prospectively with the use of standardized questionnaires that were mailed to all study subjects annually. Trained PLCO data abstracters reviewed and confirmed each reported case of cancer.

Using existing data from medical records (including clinical data, results of laboratory tests and flow cytometry, and pathology reports), we conducted an independent review of all the available clinical records for patients with CLL in this study. The study was conducted according to a protocol approved by the institutional review board at the National Cancer Institute and at each of the 10 screening centers.

HANDLING OF SAMPLES

Because standard Ficoll gradient lymphocyte separation and cryopreservation are prohibitively expensive for large epidemiologic studies, investigators in the PLCO trial developed and validated a field-collection protocol[29] involving the collection of blood at the nationwide screening centers in acid–citrate–dextrose, followed by express shipment to a processing laboratory in Frederick, Maryland, where whole-blood aliquoting with 5% dimethylsulfoxide to cryovials and controlled-rate step-freezing took place, to establish a trial-wide viable lymphocyte repository. Whole-blood samples (1 ml) that were stored in liquid nitrogen freezers were thawed and an aliquot of 500 μl was used for six-color flow cytometry (with antibodies CD45, CD19, CD5, CD10, kappa, and lambda); the remaining 500 μl of whole blood was used for analyses of the immunoglobulin heavy-chain variable (*IGHV*) gene.

FLOW CYTOMETRY

Two 100-μl samples of thawed blood were placed in two tubes for staining. They were washed three times with the use of Sorvall cell washers. Then 10 μl of rabbit immunoglobulin was added and incubated at 37°C for 30 minutes, followed by 10 μl of an isotypic cocktail (consisting of fluorescein isothiocyanate–conjugated IgG, phycoerythrin-conjugated IgG, peridinin–chlorophyll–protein cyanine 5.5–conjugated anti-CD19 antibodies, phycoerythrin–cyanine 7–conjugated IgG, allophycocyanin-conjugated IgG, and allophycocyanin H7–conjugated anti-CD45 antibodies), which was added to one tube; the second tube was stained with the custom conjugated six-color antibodies cocktail (consisting of fluorescein isothiocyanate–conjugated anti-CD5, phycoerythrin-conjugated antilambda, peridinin–chlorophyll–protein cyanine 5.5–conjugated anti-CD19 antibodies, phycoerythrin–cyanine 7–conjugated CD10, allophycocyanin-conjugated anti-kappa, and allophycocyanin H7–conjugated anti-CD45 antibodies) and incubated for 20 minutes. All antibodies were obtained from BD Biosciences. After this procedure, 1 ml of redcell lyse buffer was added to each tube and incubated for 10 minutes and then washed with Sorvall cell washer. Cells were then resuspended in 350 μl of phosphate-buffered saline and acquired with the use of FACSCanto flow cytometry; daily quality control and assurance were carried out with the use of seven-color setup beads (BD Biosciences).

IGHV ANALYSIS

Total RNA was manually extracted from the whole blood using RNA-BEE (Tel Test) and purified by Dynal magnetic beads (Invitrogen) to obtain messenger RNA (mRNA). The purified mRNA was amplified with the use of six-sense *IGHV* family-specific (HV1–HV6) leader primers and an antisense μ-chain constant-region primer in one multiplex one-step reverse-transcriptase–polymerase-chainreaction (RT-PCR) assay.

The clonal product, when present, was excised from gel, purified by the MinElute gel-extraction method (Qiagen), and sequenced with heavy-chain reverse primer with the use of BigDye Terminator, version 3.1, on a ABI 3730 Genetic analyzer (Applied Biosystems). Subcloning was used for clonal cases occurring on a polyclonal background with the use of a TOPO TA cloning kit (Invitrogen). The PCR products were ligated into the vector and transformed into *Escherichia coli* cells. Eight colonies were randomly selected for

sequencing, as described above. Nucleotide sequences were analyzed with the use of the IMGT database and tools[30] and aligned to the closest match with the germ-line *IGHV* segment. Sequences with a germ-line identity of less than 98% were considered to be mutated, whereas those with a germ-line identity of 98% or more were considered to be unmutated.[31,32]

STATISTICAL ANALYSIS

Conventional descriptive measures were used. Exact 95% binomial confidence intervals were computed for estimates of proportions.

RESULTS

PATIENTS

A total of 45 patients with CLL who had available stored prediagnostic samples of cryopreserved whole blood were included in the study (Table 1). The mean age of the patients was 70 years, and 67% of the patients were men. The median time between the time at which the prediagnostic blood sample was obtained and the subsequent CLL diagnosis was approximately 3 years (range, 3 months to 77 months). Among 43 patients with available information on Rai staging of disease (ranging from 0 [low risk] to I or II [intermediate risk] to III or IV [high risk]) at the time of the diagnosis of CLL, 40 patients (93%) had stage 0 or I, 2 patients (5%) had stage II, and 1 patient (2%) had stage III disease (Table 2).

FLOW-CYTOMETRIC AND MOLECULAR ANALYSES

As a first step, using six-color flow cytometry and a gating strategy that has been described previously,[19] we identified prediagnostic B-cell clones in 42 of 45 patients (93%) on the basis of the presence of an abnormal kappa:lambda ratio (>3:1) or negativity for both chains (Fig. 1). The results for the remaining three patients (Patients 6, 27, and 30) were indeterminate, since there were too few cells in the sample to perform a reliable assessment of clonality (Table 2). Among the 42 patients with a detectable prediagnostic clone, the B cells showed a kappa restriction in 28 patients (67%), a lambda restriction in 8 (19%), and an apparent negativity for both light chains in 3 (7%). On the basis of flow cytometry, in three patients (Patients 19, 26, and 38) (7%), the B cells showed a biclonal pattern of light-chain restriction.

As a second step, for all prediagnostic blood samples, analysis of *IGHV* gene rearrangement was performed by RT-PCR assay. A monoclonal band was demonstrated in 43 of 45 patients (96%) (Fig. 2). Patients 6 and 8 showed no evidence of monoclonality (Table 2).

As a third and last step, for all 43 patients with evidence of monoclonality on RT-PCR assay, the monoclonal rearrangement was directly sequenced and the *IGHV*-subgroup status and mutational status were assessed. We were able to identify and analyze the *IGHV* genes in 35 of 45 patients (78%), probably because of the limited number of clonal B cells (or possibly because of the presence of more than one clone with overlapping sequences) in these prediagnostic samples.[33] Among the 35 sequenced *IGHV* gene rearrangements, 16 samples

(46%) had *IGHV3*-subgroup genes and 9 (26%) had *IGHV4*-subgroup genes, frequencies that are similar to the expected frequency in the normal B-cell repertoire. In particular, the most prevalent *IGHV* genes were *IGHV3–23*, which were identified in six patients (17%), and *IGHV4–34*, which were identified in four (11%). The vast majority of the *IGHV* sequences (27 of 35, or 77%) were mutated (range of germ-line identity, 87.9 to 97.9%), whereas only 8 of the sequences (23%) were unmutated, including those in 6 samples with a 100% identity with the closest germ-line *IGHV* gene.

In summary, on the basis of either flow-cytometric or molecular analysis, we demonstrated that 44 of 45 patients with CLL (98%; 95% confidence interval [CI], 88 to 100) had a prediagnostic B-cell clone. In particular, 41 patients (91%; 95% CI, 79 to 98) had a prediagnostic clone detected by both techniques, whereas 2 patients (Patients 27 and 30) (4%) had evidence of a prediagnostic clone only on RT-PCR analysis, and 1 patient (Patient 8) (2%) had evidence of a prediagnostic clone only on six-color flow cytometry. One patient (Patient 6) had negative results on the basis of both methods; all 10 control subjects also had negative results.

PREDIAGNOSTIC PRESENCE OF B-CELL CLONE

To test whether there were differences with regard to characteristics of the observed prediagnostic clones in relation to the time between the time at which the prediagnostic blood sample was obtained and the subsequent CLL diagnosis (i.e., latency), we conducted analyses stratified according to the mean latency (≤32 months or >32 months). Among patients with latency of more than 32 months, we were able to define the presence of the *IGHV* gene and determine the mutational status in 14 of 19 patients. Of these patients, 11 (79%) had mutated genes, and 3 (21%) had unmutated genes. For patients with a latency of 32 months or less, the mutational status of *IGHV* was defined in 21 of 27 patients. Of these patients, 16 (76%) had mutated genes, and 5 (24%) had unmutated genes. The distribution of the presence of *IGHV* was similar in the two groups (Table 2). When we assessed patterns of kappa or lambda light chains of the prediagnostic clones according to mutational status, among 30 patients for whom results were available on both light-chain restriction and mutational status, we found that 19 of 25 patients with mutated genes (76%) and 4 of 5 patients with unmutated genes (80%) were kappa-positive.

LIGHT-CHAIN RESTRICTION

Using existing flow-cytometric data from medical records that were obtained at the time of the CLL diagnosis (available for 25 patients), we compared patterns of light-chain restriction at diagnosis with those observed in the prediagnostic samples and found all to be identical (Table 2). In particular, both the prediagnostic clone and the clone at CLL diagnosis showed kappa light-chain restriction in 19 patients and lambda light-chain restriction in 4 patients. For Patients 19 and 38, we found evidence of two detectable clones (i.e., biclonal light-chain restriction) in the prediagnostic sample, and at the time of CLL diagnosis, the clone was defined as kappa.

DISCUSSION

We were intrigued by recent hospital-based case series suggesting that patients with MBL are at increased risk for CLL[23,26] and wanted to test the hypothesis that CLL is typically preceded by MBL as a precursor state. Among 77,469 healthy adults who were enrolled in the nationwide, population-based PLCO Cancer Screening Trial,[27] we identified 45 patients who were subsequently diagnosed with CLL and had prospectively stored prediagnostic peripheral-blood samples, which had been obtained up to 6.4 years earlier. Using six-color flow cytometry and *IGHV* gene-rearrangement analysis by RT-PCR, we found evidence of prediagnostic monoclonality among B cells (by either of the two methods) in 44 patients (98%; 95% CI, 88 to 100). In 41 patients (91%; 95% CI, 79 to 98), the clone was confirmed by both methods.

It is well known that the mutational status of *IGHV* carries a strong prognostic value in CLL, with patients carrying unmutated genes having a more aggressive course of the disease, as reflected in a shorter life expectancy.[31,32] Despite the limited size of the prediagnostic clones and limited availability of specimens, we were able to define *IGHV* mutational status for 35 of 45 clones, demonstrating that the vast majority (approximately 80%) carried mutated *IGHV* genes. This finding notwithstanding, patients with unmutated genes were also observed, some with 100% identity with the closest germ-line genes. The distribution of mutated clones, as compared with unmutated clones, was very similar regardless of the time between the time at which the blood sample was obtained and the subsequent CLL diagnosis. In addition, among eight unmutated prediagnostic clones, three were present more than 3 years before the CLL diagnosis, with two being detectable 5 years before.

Our findings show that virtually all cases of CLL (both with mutated genes and unmutated genes) are preceded by MBL. It is also interesting to note that both indolent disease (stage 0 or I) and more aggressive disease (stage II or III) showed evidence of a prediagnostic clone up to 3 years before the diagnosis of CLL. However, because the distribution of unmutated genes, as compared with mutated genes, was somewhat lower (23%) than distributions reported previously (35 to 40%),[34] one might speculate that there are more aggressive CLL cases that present de novo without being preceded by MBL. Alternatively, our findings might reflect the ratio between unmutated and mutated genes in a nonclinic-based population of patients with CLL.

The strengths of our study include its unique population-based, prospective design, the availability of prediagnostic blood samples, and the application of high-quality assays for the determination of the presence of prediagnostic B-cell clones. The study also had some limitations. Because of the design of the PLCO Cancer Screening Trial, blood samples at the time of CLL diagnosis were not available. To partially address this issue, we used existing clinical data regarding light-chain restriction in CLL diagnostic clones. When we compared the CLL clone with the prediagnostic clone of the same patient, we did not find any cases in which either kappa or lambda expression was discordant. These findings are consistent with the contention that the prediagnostic clone subsequently evolved into clinically detectable CLL. It may also be questioned whether the high frequency of MBL before CLL diagnosis represents, in part, a manifestation of undetected leukemia present months to years before

the manifestations of CLL. However, for the majority of patients in our study, we believe this hypothesis is unlikely. The PLCO population is relatively health-conscious and has volunteered for and complied with the requirements of a screening trial, including annual screening tests and blood collection. This is reflected in the very high proportion of early-stage CLL among patients in our study.

Although screening for MBL outside a research study is not recommended, its incidental detection will continue. Physicians need to be aware that the diagnosis of MBL may lead to unneeded distress and increased health care costs.[35] Taken together, these facts emphasize the need for future population-based and preclinical studies designed to improve our understanding of the progression of leukemogenesis, particularly in the early window before the development of clinical CLL.[1,36]

In conclusion, we found that the vast majority of patients with CLL have a precursor state from 6 months to 6 years before the development of clinically recognized leukemia. Future studies are needed to provide insights into the pathogenesis of MBL and predictors for CLL progression. A better description of mechanisms mediating B-cell clonal proliferation and survival will ultimately enhance our understanding of the pathophysiology of MBL and CLL and allow for the identification of new molecular targets.

Acknowledgments

Supported by the Intramural Research Program of the National Cancer Institute.

Dr. Ghia reports receiving grant support from the Italian Association for Cancer Research and the CLL Global Research Foundation; and Dr. Albitar and Ms. Ma, being employees of Quest Diagnostics. No other potential conflict of interest relevant to this article was reported.

We thank Christine Berg and Philip Prorok of the Division of Cancer Prevention, National Cancer Institute; the Screening Center investigators and staff of the PLCO Cancer Screening Trial; Tom Riley and the staff of Information Management Services; Barbara O'Brien and the staff of Westat; Ruth Pfeiffer of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, for statistical help; Kostas Stamatopoulos of Thessaloniki, Greece, for his most helpful advice and suggestions; and the patients who made this study possible.

References

1. Linet MS, Schubauer-Berigan MK, Weisenburger DD, et al. Chronic lymphocytic leukaemia: an overview of aetiology in light of recent developments in classification and pathogenesis. Br J Haematol 2007;139:672–86. [PubMed: 18021081]
2. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. World Health Organization classification of tumours Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press, 2001.
3. Goldin LR, Bjorkholm M, Kristinsson SY, Turesson I, Landgren O. Elevated risk of chronic lymphocytic leukemia (CLL) and other indolent non-Hodgkin lymphomas among relatives of CLL patients. Haematologica 2009 (in press).
4. Ries LAG, Melbert D, Krapcho M, et al., eds. SEER cancer statistics review, 1975–2005 (based on November 2007 SEER data submission). Bethesda, MD: National Cancer Institute, 2008.
5. Jamroziak K, Balcerczak E, Smolewski P, et al. MDR1 (ABCB1) gene polymorphism C3435T is associated with P-glycoprotein activity in B-cell chronic lymphocytic leukemia. Pharmacol Rep 2006;58:720–8. [PubMed: 17085864]
6. Rudd MF, Sellick GS, Webb EL, Catovsky D, Houlston RS. Variants in the ATM-BRCA2-CHEK2 axis predispose to chronic lymphocytic leukemia. Blood 2006; 108:638–44. [PubMed: 16574953]

7. Thorsélius M, Kröber A, Murray F, et al. Strikingly homologous immunoglobulin gene rearrangements and poor outcome in VH3–21-using chronic lymphocytic leukemia patients independent of geographic origin and mutational status. Blood 2006;107:2889–94. [PubMed: 16317103]
8. Messmer BT, Albesiano E, Messmer D, Chiorazzi N. The pattern and distribution of immunoglobulin VH gene mutations in chronic lymphocytic leukemia B cells are consistent with the canonical somatic hypermutation process. Blood 2004;103:3490–5. [PubMed: 14695232]
9. Carbone A, Gloghini A, Gaidano G, et al. Expression status of BCL-6 and syndecan-1 identifies distinct histogenetic subtypes of Hodgkin's disease. Blood 1998; 92:2220–8. [PubMed: 9746758]
10. Stamatopoulos K, Belessi C, Moreno C, et al. Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: pathogenetic implications and clinical correlations. Blood 2007;109:25970.
11. Landgren O, Engels EA, Caporaso NE, et al. Patterns of autoimmunity and subsequent chronic lymphocytic leukemia in Nordic countries. Blood 2006;108:292–6. [PubMed: 16527887]
12. Landgren O, Gridley G, Check D, Caporaso NE, Morris Brown L. Acquired immune-related and inflammatory conditions and subsequent chronic lymphocytic leukaemia. Br J Haematol 2007;139:791–8. [PubMed: 17941950]
13. Landgren O, Rapkin JS, Caporaso NE, et al. Respiratory tract infections and subsequent risk of chronic lymphocytic leukemia. Blood 2007;109:2198–201. [PubMed: 17082317]
14. French Cooperative Group on Chronic Lymphocytic Leukaemia. Natural history of stage A chronic lymphocytic leukaemia untreated patients. Br J Haematol 1990; 76:45–57.
15. Han T, Ozer H, Gavigan M, et al. Benign monoclonal B cell lymphocytosis — a benign variant of CLL: clinical, immunologic, phenotypic, and cytogenetic studies in 20 patients. Blood 1984;64:244–52. [PubMed: 6610446]
16. Garnier JL, Lebranchu Y, Lefrancois N, et al. Hodgkin's disease after renal transplantation. Transplant Proc 1995;27:1785. [PubMed: 7725504]
17. Marti GE, Rawstron AC, Ghia P, et al. Diagnostic criteria for monoclonal B-cell lymphocytosis. Br J Haematol 2005;130: 325–32. [PubMed: 16042682]
18. Montserrat E, Vinolas N, Reverter JC, Rozman C. Natural history of chronic lymphocytic leukemia: on the progression and progression and prognosis of early clinical stages. Nouv Rev Fr Hematol 1988; 30:359–61. [PubMed: 3222145]
19. Rawstron AC, Green MJ, Kuzmicki A, et al. Monoclonal B lymphocytes with the characteristics of "indolent" chronic lymphocytic leukemia are present in 3.5% of adults with normal blood counts. Blood 2002;100:635–9. [PubMed: 12091358]
20. Vogt RF, Meredith MNK, Powell J, et al. Results in eleven individuals with B-CLL-like phenotypes detected in environmental health studies In: Marti GE, Vogt RF, Zenger VE, eds. Proceedings of the USPHS workshop on laboratory approaches to determining the role of environmental exposures as risk factors for B-cell chronic lymphocytic leukemia and other B-cell lymphoproliferative disorders. Atlanta: Department of Health and Human Services, 1995:19–35.
21. Tomita Y, Ohsawa M, Kanno H, et al. Epstein-Barr virus in Hodgkin's disease patients in Japan. Cancer 1996;77:186–92. [PubMed: 8630928]
22. Ghia P, Prato G, Scielzo C, et al. Monoclonal CD5+ and CD5- B-lymphocyte expansions are frequent in the peripheral blood of the elderly. Blood 2004;103:2337–42. [PubMed: 14630808]
23. Rawstron AC, Bennett FL, O'Connor SJ, et al. Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. N Engl J Med 2008;359:575–83. [PubMed: 18687638]
24. Rawstron AC. Prevalence and characteristics of monoclonal B-cell lymphocytosis (MBL) in healthy individuals and the relationship with clinical disease. J Biol Regul Homeost Agents 2004;18:155–60. [PubMed: 15471220]
25. de Tute R, Yuille M, Catovsky D, Houlston RS, Hillmen P, Rawstron AC. Monoclonal B-cell lymphocytosis (MBL) in CLL families: substantial increase in relative risk for young adults. Leukemia 2006;20:728–9. [PubMed: 16437141]
26. Rawstron AC, Bennett F, Hillmen P. The biological and clinical relationship between CD5+23+ monoclonal B-cell lymphocytosis and chronic lymphocytic leukaemia. Br J Haematol 2007;139:724–9. [PubMed: 18021087]

27. Prorok PC, Andriole GL, Bresalier RS, et al. Design of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Control Clin Trials 2000;21: Suppl:273S–309S. [PubMed: 11189684]

28. Hayes RB, Sigurdson A, Moore L, et al. Methods for etiologic and early marker investigations in the PLCO trial. Mutat Res 2005;592:147–54. [PubMed: 16054167]

29. Hayes RB, Smith CO, Huang WY, Read Y, Kopp WC. Whole blood cryopreservation in epidemiological studies. Cancer Epidemiol Biomarkers Prev 2002;11:1496–8. [PubMed: 12433734]

30. Giudicelli V, Chaume D, Lefranc MP. IMGT/V-QUEST, an integrated software program for immunoglobulin and T cell receptor V-J and V-D-J rearrangement analysis. Nucleic Acids Res 2004;32:W435–W440. [PubMed: 15215425]

31. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94: 1840–7. [PubMed: 10477712]

32. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999;94:1848–54. [PubMed: 10477713]

33. Ghia P, Stamatopoulos K, Belessi C, et al. ERIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukemia. Leukemia 2007;21:1–3. [PubMed: 17167526]

34. Kipps TJ. The B-cell receptor and ZAP-70 in chronic lymphocytic leukemia. Best Pract Res Clin Haematol 2007;20:415–24. [PubMed: 17707830]

35. Shanafelt TD, Kay NE, Call TG, et al. MBL or CLL: which classification best categorizes the clinical course of patients with an absolute lymphocyte count > or =5×10(9)L(−1) but a B-cell lymphocyte count <5×10(9)L(−1)? Leuk Res 2008;32:1458–61. [PubMed: 18179821]

36. Landgren O, Kyle RA. Multiple myeloma, chronic lymphocytic leukaemia and associated precursor diseases. Br J Haematol 2007;139:717–23. [PubMed: 18021086]

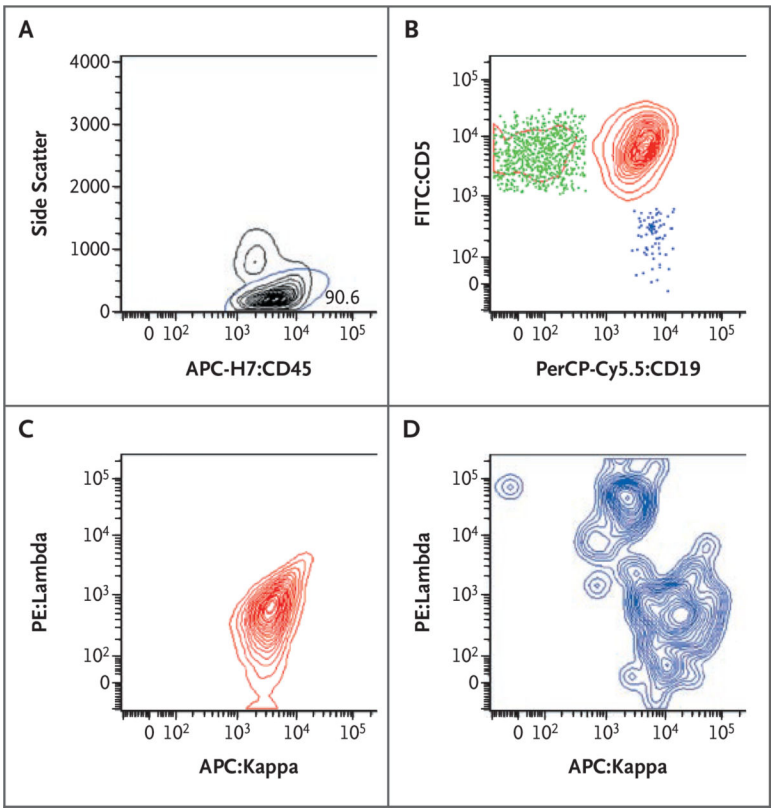

Figure 1. Six-Color Flow-Cytometric Analysis, Showing Representative Kappa Light-Chain Restriction in Prediagnostic Blood Samples from the Patients.

Panel A shows a bivariant plot of CD45 against side scatter, showing the isolation of lymphocytes incubated with anti-CD45 allophycocyanin H7 (APC-H7). Panel B shows a plot of CD19 against CD5. The monoclonal B-cell lymphocytosis (MBL) clone (red), positive for anti-CD19 peridinin–chlorophyll–protein cyanine 5.5 (PerCP-Cy5.5), shows coexpression of CD19 and CD5. The green population represents the remaining CD5-positive T cells, and the blue population represents the remaining polyclonal B cells. Panel C shows that the red population, positive for anti-kappa allophycocyanin (APC), has a kappa light-chain restriction. Panel D shows that the remaining B cells (blue) are polyclonal. FITC denotes fluorescein isothiocyanate, and PE phycoerythrin.

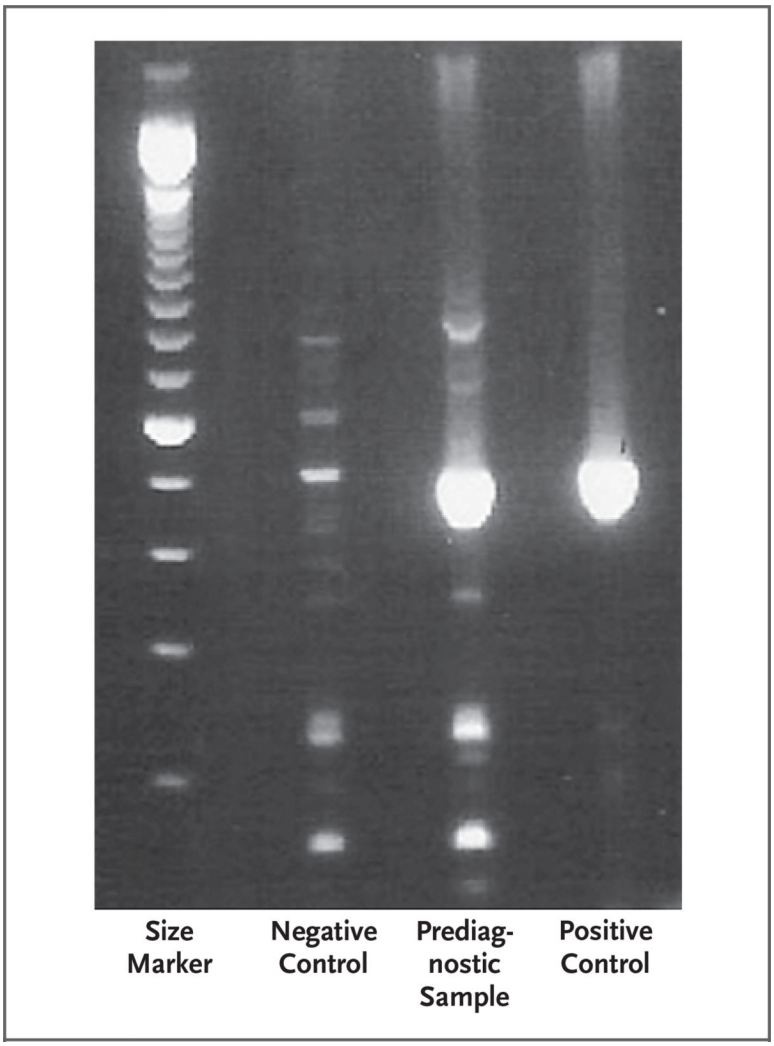

Figure 2.
IGHV Rearrangements, as Detected on RT-PCR Assay, Showing a Monoclonal Band in a Prediagnostic Blood Sample from a Patient with Subsequent CLL.

Table 1.

Characteristics of the Patients.*

Variable	Value
Total no. of patients	45
Age at CLL diagnosis — yr	
Mean	70
Range	61–79
Male sex — no. (%)	30 (67)
Race — no. (%)†	
White	42 (93)
Black	3 (7)
Calendar year of CLL diagnosis	
Median	2002
Range	1999–2005
Time between collection of the prediagnostic blood sample and the subsequent CLL diagnosis — mo	
Mean	32
Range	3–77

*CLL denotes chronic lymphocytic leukemia.

†Race was self-reported.

Table 2.

Characteristics of Prediagnostic Blood Samples of Patients with a Subsequent CLL Diagnosis. *

Patient Number	Prediagnostic Blood Sample								At CLL Diagnosis					
	Flow Cytometry†					RT-PCR	*IGHV*		Flow Cytometry			Absolute Lymphocyte Count	Rai Stage§	Latency¶
	Light-Chain Restriction	Lymphocytes	CD19+	CD19/5+	CD5 Exp		Identity‡	Subgroup	Light-Chain Restriction	ZAP-70	CD38+			
			%				%					*per mm*^{3}		*mo*
1	K	25.2	22.7	26.5	Intermediate	Positive	100.0‖	5–51	K	Positive	7% Positive	33,580	0	77
2	K	21.5	29.5	53.5	Bright	Positive	97.9	3–23	NA	NA	NA	7,020	I	72
3	K	4.32	46.3	82	Intermediate	Positive	93.1	3–23	K	NA	NA	NA	NA	72
4	K	21.6	30.5	47	Bright	Positive	96.3	6–1	K	NA	NA	15,000	0	61
5	K	30	76.6	96	Bright	Positive	94.4	4–34	K	NA	NA	NA	0	58
6	IM	0.86	53.3	12.5	IN	Negative	NA	NA	L	Positive	NA	29,727	II	54
7	K	64	82.2	98.2	IN	Positive	100.0‖	4–31	NA	NA	Positive **	189,750	I	54
8	K	13.5	22.4	16.4	Negative	Negative	NA	NA	K	NA	NA	NA	0	48
9	K	12.7	89.3	38	Intermediate	Positive	95.1	1–18	K	NA	NA	14,288	0	46
10	L	82	94.7	98.8	Intermediate	Positive	95.5	2–5	L	NA	Negative	NA	NA	44
11	K	66	81.8	79.3	Intermediate	Positive	NA	NA	K	NA	NA	6,669	0	44
12	K	70.5	51.5	87.6	Intermediate	Positive	93.4	3–7	K	NA	11% Positive	10,280	0	43
13	L	46.9	48.1	96	Bright	Positive	91.2	4–61	L	NA	NA	7,523	0 or I	41
14	K	36.3	6.76	52.6	Intermediate	Positive	96.6	3–49	NA	NA	NA	10,600	0 or I	40
15	K	21.6	48.9	91	Bright	Positive	NA	NA	K	NA	NA	10,251	0	39
16	L	44.8	24.9	31.1	Intermediate	Positive	88.5	3–23	NA	NA	NA	11,780	III	38
17	K	50.3	62.4	90.4	Intermediate	Positive	100.0‖	3–7	K	NA	Positive **	12,650	0	37
18	L	66.1	80.1	98.4	Intermediate	Positive	NA	NA	L	NA	43% Positive	22,500	I	36
19	Biclonal	47.8	93.4	27	Bright	Positive	96.5	4–34	K	NA	Positive **	14,688	I	34

Patient Number	Prediagnostic Blood Sample								At CLL Diagnosis					
	Flow Cytometry†					RT-PCR	*IGHV*		Flow Cytometry			Absolute Lymphocyte Count	Rai Stage§	Latency¶
	Light-Chain Restriction	Lymphocytes	CD19+	CD19/5+	CD5 Exp		Identity‡	Subgroup	Light-Chain Restriction	ZAP-70	CD38+			
			%				%					*per mm*^{3}		*mo*
20	K	39.3	84.1	99.2	Bright	Positive	90.3	3–30	NA	NA	NA	19,716	II	31
21	K	16.4	66.9	80.2	Intermediate	Positive	NA	NA	K	NA	NA	NA	0	31
22	K	33.5	82.2	92.8	Bright	Positive	88.9	3–23	NA	NA	NA	NA	0	29
23	K	59.9	81.9	98.1	Bright	Positive	88.4	3–23	NA	NA	NA	17,430	0 or I	28
24	K	0.94	55.9	59	Intermediate	Positive	92.4	3–23	K	NA	NA	6,588	0	26
25	IN	44.6	91.9	99.1	Intermediate	Positive	NA	NA	K	NA	NA	13,400	I	26
26	Biclonal	47.3	37	87.4	Bright	Positive	91.7	5–51	NA	NA	NA	NA	0 or I	25
27	IM	16.8	61.3	58	IN	Positive	100.0//	1–3	K	NA	NA	43,860	0 or I	25
28	L	35.3	83.6	98.9	Intermediate	Positive	93.7	3–53	NA	NA	NA	10,877	0	25
29	IN	68.3	74.9	98.4	Intermediate	Positive	93.5	2–5	L	90% Positive	NA	50,776	0	24
30	IM	0.58	7.3	25	IN	Positive	99.3//	3–33	NA	NA	NA	10,506	1	24
31	L	1.64	77.9	97.9	Intermediate	Positive	97.6	3–48	NA	NA	NA	NA	I	21
32	K	43	63.7	96.3	Bright	Positive	100.0//	4–39	K	NA	25% Positive	88,810	0	21
33	L	85.9	98	97.5	Intermediate	Positive	100.0//	2–5	L	NA	28% Positive	44,268	I	20
34	L	24.7	77.8	95.2	Bright	Positive	NA	NA	NA	NA	Negative	13,000	0	20
35	K	58.4	91.6	73.2	Intermediate	Positive	NA	NA	K	NA	37% Positive	7,442	0	19
36	K	12.9	98.5	99.3	Bright	Positive	96.1	4–34	K	NA	NA	17,900	0	18
37	K	90.7	93.2	99.5	Bright	Positive	90.9	4–34	NA	NA	NA	14,620	0	13
38	Biclonal	68.7	77.9	75.1	Bright	Positive	NA	NA	K	NA	NA	95,880	0	13
39	K	13.7	75.5	98.3	Bright	Positive	87.9	4–39	K	NA	NA	13,460	0	12
40	K	45	79.5	98.2	Bright	Positive	94.8	3–33	K	NA	NA	5,609	0	11
41	K	87.4	94	98.2	Bright	Positive	92.0	1–46	K	NA	NA	15,444	0	10

N Engl J Med. Author manuscript; available in PMC 2020 February 12.

Patient Number	Prediagnostic Blood Sample								At CLL Diagnosis					
	Flow Cytometry†					RT-PCR	*IGHV*		Flow Cytometry			Absolute Lymphocyte Count	Rai Stage§	Latency¶
	Light-Chain Restriction	Lymphocytes	CD19+	CD19/5+	CD5 Exp		Identity‡	Subgroup	Light-Chain Restriction	ZAP-70	CD38+			
		%					%					*per mm*^{3}		*mo*
42	K	71.4	84.9	26.2	Dim	Positive	91.3	3–23	NA	NA	Negative	12,320	0	8
43	K	80.8	94.2	99.3	Intermediate	Positive	95.6	3–72	K	NA	NA	35,155	I	7
44	K	1.28	83.5	67.5	Intermediate	Positive	96.2	3–11	NA	NA	Negative	15,812	0	4
45	IN	8.04	82.3	98.8	Intermediate	Positive	99.0‖	1–69	K	Negative	Negative	80,259	0	3

*CD5 Exp denotes CD5-expressing B cells, CLL chronic lymphocytic leukemia, IGHV immunoglobulin heavy-chain variable, IM insufficient material, IN indeterminate, K kappa, L lambda, NA not available, and RT-PCR reverse-transcriptase polymerase chain reaction.

†Listed are percentages of lymphocytes among all cells in the sample, percentages of CD19+ B cells among all lymphocytes, and percentages of CD19/5+ B cells among all CD19+ B cells.

‡Nucleotide sequences were analyzed with the use of the IMGT database and tools[30] and were aligned to the closest match with a germ-line *IGHV* segment. Sequences with a germ-line identity of less than 98% were considered to be mutated, whereas those with a germ-line identity of 98% or more were considered to be unmutated.[31,32]

§The Rai staging system ranges from 0 (low risk) to I or II (intermediate risk) to III or IV (high risk). The Rai stage was defined to the degree possible on the basis of available clinical and laboratory data in each patient's medical record. For five patients, it could not be determined whether the Rai stage was 0 or I.

¶Latency refers to the time in months between the collection of the prediagnostic blood sample and the subsequent CLL diagnosis.

‖These patients had unmutated *IGHV* genes.

**Data regarding the percentage of CD38+ cells were missing.